Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1. (currently amended) A recombinant fluorescent protein having the ~~following~~ amino acid ~~sequences~~ sequence ~~(1) to (3)~~ in order in the direction from the N-terminus to the C-terminus, ~~wherein a fused fluorescent protein obtained by fusion of the fluorescent protein with a calcium binding protein and its target peptide can emit fluorescence which is dependent on Ca2+ ion level;~~

~~(1)~~ (a) an amino acid sequence from the n^{th} amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

~~(2)~~ (b) a linker sequence of a sequence of 2 to 20 amino acids; and

~~(3)~~ (c) an amino acid sequence from the 1st amino acid to the (n-1)th amino acid from the N-terminus of the fluorescent protein described in ~~(1)~~ (a) above,

wherein the fused fluorescent protein obtained by fusion of the fluorescent protein with a calcium binding protein and its target peptide can emit fluorescence which is dependent on Ca2+ ion level.

2. (original) The fluorescent protein according to claim 1 wherein, under the presence of Ca^{2+} ions, the fused fluorescent protein can emit fluorescence of an intensity which differs depending on Ca^{2+} ion level.

3. (original) The fluorescent protein according to claim 1 wherein, under the presence of Ca^{2+} ions, the fused fluorescent protein can show excitation of a wavelength which differs depending on Ca^{2+} ion level.

4. (currently amended) The fluorescent protein according to claim 1 wherein the amino acid sequence of a linker sequence is ~~Gly-Gly-Ser-Gly-Gly~~ the sequence set forth in SEQ ID NO:4 or ~~Val-Asp-Gly-Gly-Ser-Gly-Gly-Thr-Gly~~ the sequence set forth in SEQ ID NO:5.

5. (currently amended) A recombinant fluorescent protein of any of the followings;

(A) a protein having the amino acids from position 32 to 275 of the amino acid sequence shown in ~~SEQ ID NO:1~~ SEQ ID NO:6, or a protein having an amino acid sequence having a deletion, substitution and/or addition of one to several amino acids in the amino acids from position 32 to 275 of the amino acid sequence shown in ~~SEQ ID NO:1~~ SEQ ID NO:6 and having a fluorescence characteristic that is equivalent to or greater than that of a protein having the amino acids from position 32 to 275 of the amino acid sequence shown in ~~SEQ ID NO:1~~ SEQ ID NO:6;

(B) a protein having the amino acids from position 32 to 278 of the amino acid sequence shown in ~~SEQ ID NO:2~~ SEQ ID NO:7, or a protein having an amino acid sequence having a deletion, substitution and/or addition of one to several amino acids in the amino acids from position 32 to 278 of the amino acid sequence shown in ~~SEQ ID NO:2~~ SEQ ID NO:7 and having a fluorescence characteristic that is equivalent to or greater than that of a protein having the amino acids from position 32 to 278 of the amino acid sequence shown in ~~SEQ ID NO:2~~ SEQ ID NO:7; or,

(C) a protein having the amino acids from position 32 to 278 of the amino acid sequence shown in ~~SEQ ID NO:3~~ SEQ ID NO:8, or a protein having an amino acid sequence having a deletion,

substitution and/or addition of one to several amino acids in the amino acids from position 32 to 278 of the amino acid sequence shown in ~~SEQ ID NO:3~~ SEQ ID NO:8 and having a fluorescence characteristic that is equivalent to or greater than that of a protein having the amino acids from position 32 to 278 of the amino acid sequence shown in ~~SEQ ID NO:3~~ SEQ ID NO:8.

6. (currently amended) A fused fluorescent protein which can emit fluorescence that is dependent on Ca^{2+} ion level having the following amino acid sequences ~~(1) to (5)~~ (a) to (e) in order in the direction from the N-terminus to the C-terminus, ~~which can emit fluorescence that is dependent on Ca2+ ion level.~~

~~(1)~~ (a) an amino acid sequence of a target peptide of a calcium-binding protein;

~~(2)~~ (b) an amino acid sequence from the n^{th} amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

~~(3)~~ (c) a linker sequence of a sequence of 2 to 20 amino acids;

~~(4)~~ (d) an amino acid sequence from the 1st amino acid to the (n-1)th amino acid from the N-terminus of the fluorescent protein described in ~~(2)~~ (b) above; and

~~(5)~~ (e) the amino acid sequence of a calcium-binding protein.

7. (original) The fused fluorescent protein according to claim 6 which can, under the presence of Ca^{2+} ions, emit fluorescence of an intensity that differs depending on Ca^{2+} ion level.

8. (original) The fused fluorescent protein according to claim 6 which can, under the presence of Ca^{2+} ions, show excitation of a wavelength that differs depending on Ca^{2+} ion level.

9. (currently amended) The fused fluorescent protein according to claim 6 wherein the amino acid sequence of a linker sequence is ~~Gly-Gly-Ser-Gly-Gly~~ the sequence set forth in SEQ ID NO:4 or ~~Val-Asp-Gly-Gly-Ser-Gly-Gly-Thr-Gly~~ the sequence set forth in SEQ ID NO:5.

10. (original) The fused fluorescent protein according to claim 6 wherein the calcium-binding protein is a protein selected from the group consisting of: calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins, parvalbumin, calbindin D9K, calbindin D28K, and calretinin.

11. (currently amended) A fused fluorescent protein of any of the followings;

(A) a protein having the amino acid sequence shown in ~~SEQ ID NO:1~~ SEQ ID NO:6, or a protein having an amino acid sequence having a deletion, substitution and/or addition of one to several amino acids in the amino acid sequence shown in ~~SEQ ID NO:1~~ SEQ ID NO:6 and having a fluorescence characteristic that is equivalent to or greater than that of a protein having the amino acid sequence shown in ~~SEQ ID NO:1~~ SEQ ID NO:6;

(B) a protein having the amino acid sequence shown in ~~SEQ ID NO:2~~ SEQ ID NO:7, or a protein having an amino acid sequence having a deletion, substitution and/or addition of one to several amino acids in the amino acid sequence shown in ~~SEQ ID NO:2~~ SEQ ID NO:7 and having a fluorescence characteristic that is equivalent to or greater than that of a protein having the amino acid sequence shown in ~~SEQ ID NO:2~~ SEQ ID NO:7; or,

(C) a protein having the amino acid sequence shown in ~~SEQ ID NO:3~~ SEQ ID NO:8, or a protein having an amino acid sequence having a deletion, substitution and/or addition of one to several amino acids in the amino acid sequence shown in ~~SEQ ID NO:3~~ SEQ ID NO:8 and having a fluorescence characteristic that is equivalent to or greater than that of a protein having the amino acid sequence shown in ~~SEQ ID NO:3~~ SEQ ID NO:8.

12. (original) A calcium ion indicator which comprises the fused fluorescent protein of claim 6.

13. (original) A method for measuring concentration or distribution of intracellular calcium ion by using the fused fluorescent protein of claim 6.

14. (original) DNA encoding the fluorescent protein of claim 1.

15. (original) DNA of any of the followings;

(A) DNA having the nucleotide sequence from position 94 to 825 of the nucleotide sequence shown in SEQ ID NO:1, or DNA having a nucleotide sequence having a deletion, substitution and/or addition of one to several nucleotides in the nucleotide sequence from position 94 to 825 of the nucleotide sequence shown in SEQ ID NO:1 and encoding a protein having a fluorescence characteristic that is equivalent to or greater than that of a protein encoded by DNA having the nucleotide sequence from position 94 to 825 of the nucleotide sequence shown in SEQ ID NO:1;

(B) DNA having the nucleotide sequence from position 94 to 834 of the nucleotide sequence shown in SEQ ID NO:2, or DNA having a nucleotide sequence having a deletion, substitution and/or addition of one to several nucleotides in the nucleotide sequence from position 94 to 834 of the nucleotide sequence shown in SEQ ID NO:2 and encoding a protein having a fluorescence

characteristic that is equivalent to or greater than that of a protein encoded by DNA having the nucleotide sequence from position 94 to 834 of the nucleotide sequence shown in SEQ ID NO:2; or,

(C) DNA having the nucleotide sequence from position 94 to 834 of the nucleotide sequence shown in SEQ ID NO:3, or DNA having a nucleotide sequence having a deletion, substitution and/or addition of one to several nucleotides in the nucleotide sequence from position 94 to 834 of the nucleotide sequence shown in SEQ ID NO:3 and encoding a protein having a fluorescence characteristic that is equivalent to or greater than that of a protein encoded by DNA having the nucleotide sequence from position 94 to 834 of the nucleotide sequence shown in SEQ ID NO:3.

16. (original) DNA encoding the fused fluorescent protein of claim 6.

17. (original) DNA of any of the followings:

(A) DNA having the nucleotide sequence shown in SEQ ID NO:1 or DNA having a nucleotide sequence having a deletion, substitution and/or addition of one to several nucleotides in the nucleotide sequence shown in SEQ ID NO:1 and encoding a protein having a fluorescence characteristic that is equivalent to or greater than that of a protein encoded by the nucleotide sequence shown in SEQ ID NO:1;

(B) DNA having the nucleotide sequence shown in SEQ ID NO:2, or DNA having a nucleotide sequence having a deletion, substitution and/or addition of one to several nucleotides in the nucleotide sequence shown in SEQ ID NO:2 and encoding a protein having a fluorescence characteristic that is equivalent to or greater than that of a protein encoded by the nucleotide sequence shown in SEQ ID NO:2; or

(C) DNA having the nucleotide sequence shown in SEQ ID NO:3, or DNA having a nucleotide sequence having a deletion, substitution and/or addition of one to several nucleotides in the nucleotide sequence shown in SEQ ID NO:3 and encoding a protein having a fluorescence characteristic that is equivalent to or greater than that of a protein encoded by the nucleotide sequence shown in SEQ ID NO:3.

18. (original) A recombinant vector having the DNA of claim 14.

19. (currently amended) A transformant having the DNA of claim 14 ~~or the recombinant vector of claim 18~~.

20. (original) A method for measuring the calcium ion concentration or distribution in the transformant of claims 19 by using the fluorescence emitted by the transformant as an index.

21. (currently amended) A kit for measuring calcium ions which comprises at least one or more components selected from

a recombinant fluorescent protein having the following amino acid ~~sequences~~ sequence ~~(1) to (3)~~ in order in the direction from the N-terminus to the C-terminus, ~~wherein a fused fluorescent protein obtained by fusion of the fluorescent protein with a calcium binding protein and its target peptide can emit fluorescence which is dependent on Ca2+ ion level;~~

~~(1)~~ (a) an amino acid sequence from the n^{th} amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

~~(2)~~ (b) a linker sequence of a sequence of 2 to 20 amino acids; and

~~(3)~~ (c) an amino acid sequence from the 1st amino acid to the (n-1)th amino acid from the N-terminus of the fluorescent protein described in ~~(1)~~ (a) above,

wherein the fused fluorescent protein obtained by fusion of the fluorescent protein with a calcium binding protein and its target peptide can emit fluorescence which is dependent on Ca^{2+} ion level, or

a fused fluorescent protein which can emit fluorescence that is dependent on Ca^{2+} ion level having the following amino acid ~~sequences~~ sequence ~~(1) to (5)~~ in order in the direction from the N-terminus to the C-terminus, ~~which can emit fluorescence that is dependent on Ca^{2+} ion level~~.

~~(1)~~ (d) an amino acid sequence of a target peptide of a calcium-binding protein;

~~(2)~~ (e) an amino acid sequence from the n^{th} amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

~~(3)~~ (f) a linker sequence of a sequence of 2 to 20 amino acids;

~~(4)~~ (g) an amino acid sequence from the 1^{st} amino acid to the $(n-1)^{th}$ amino acid from the N-terminus of the fluorescent protein described in ~~(2)~~ (e) above; and

~~(5)~~ (h) the amino acid sequence of a calcium-binding protein;

a calcium ion indicator comprising the fused fluorescent protein;

a DNA encoding the fluorescent protein;

a recombinant vector having DNA encoding the fused fluorescent protein; or

a transformant having the DNA or the recombinant vector.